



### A16 Development of a One-Step Cell Elution and Preferential Lysis Method for Analysis of Sexual Assault Samples

Jenny A. Lounsbury, MSFS\*, Jessica V. Norris, MSc, and Helina Cunniffe, BSc, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904; Robert C. Giles, PhD, Orchid Cellmark, Incorporated, 13988 Diplomat Drive, Suite 100, Farmers Branch, TX 75234; and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904

After attending this presentation, attendees will gain understanding of the improvements that have been made to the elution of cellular material from cotton swab samples collected from sexual assault victims and preferential lysis of epithelial cells recovered from the swab.

This presentation will impact the forensic community by describing an alternative, faster method, for increased cell recovery and comprehensive epithelial cell lysis from a vaginal cotton swab, than conventional differential extraction, providing the potential to shorten analysis times for sexual assault samples.

Differential extraction is a widely accepted method for the separation of male and female cellular material recovered from sexual assault swabs in order to obtain profiles of both the victim and the perpetrator. Differential extraction is laborious and time consuming, sometimes requiring an overnight incubation of the swab. This method utilizes proteinase K and a detergent to elute the majority of cellular material and selectively lyse epithelial cells. While this method has been shown to comprehensively lyse epithelial cells, some sperm cells are lost due to proteolytic digestion, thus decreasing the probability of obtaining a profile of the perpetrator in subsequent STR analysis.<sup>[1]</sup>

The use of microdevices for forensic analysis provides rapid, cost-effective alternatives to traditional DNA analysis methods. In addition, DNA analysis steps can be integrated onto one microfluidic device which will reduce analysis time and, as a result, the current backlog of casework samples. The separation of sperm cells and female epithelial cell lysate using a microfluidic device has been recently demonstrated<sup>[2]</sup>; however, this method is dependent on the recovery from the cotton swab of sperm cells as well as comprehensive lysis of epithelial cells from the vaginal lining.

Previous studies have shown that anionic detergents considerably increase the percent recovery of sperm cells over a conventional DE buffer.<sup>[1]</sup> A two step method for cell elution and preferential lysis of epithelial cells has recently been presented, which first elutes the cellular material then preferentially lyses the female epithelial cells while still obtaining high recovery of sperm cells.<sup>[3]</sup> This method uses a thirty minute incubation for cell elution and a second thirty minute incubation for preferential lysis of the epithelial cells. Using the results obtained from these studies, a one-step cell elution and preferential lysis method has been developed, which uses an anionic detergent, proteinase K, and an Orchid Cellmark reagent in the elution buffer. This improved method leads to cleaner slides for sperm counting and aids in the production of cleaner male profiles following DNA extraction.

Current work focuses on optimizing this one-step cell elution and preferential lysis method for sexual assault swabs. Several elution and lysis buffer components were evaluated along with different incubation conditions to determine the optimal recovery conditions. The sperm and epithelial cells eluted from mock sexual assault evidence swabs were counted using a hemacytometer. The results indicate that this one step method has comparable sperm cell recovery to the previous two step method and utilizes a much shorter incubation period, as little as thirty minutes. This method could be used in conjunction with conventional DNA analysis steps or eventually integrated in a lab-on-a-chip device for total DNA analysis.

#### References:

- <sup>1</sup> Norris, JV, Manning K, Linke SJ, Ferrance JP, Landers JP. *J Forensic Sci* 2007;52(4):800-805.
- <sup>2</sup> Norris, JV, Evander, M, Horsman-Hall, K, Nilsson, J, Laurell, T, Landers, JP., in preparation.
- <sup>3</sup> Norris, JV, Cunniffe, H, Manning, K, Linke, SJ, Ferrance, JP, Landers, JP. Development of an Improved Cell Elution and Preferential Lysis Method for Sexual Assault Cotton Swab Evidence Analysis; Washington, DC. American Academy of Forensic Sciences, 2008 Feb 18-23.

#### Cell Elution, Preferential Lysis, Differential Extraction